Multiple sclerosis may disrupt endocannabinoid brain protection mechanism

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Since the discovery of the endocannabinoids [eCB; anandamide and 2-arachidonoylglycerol (2-AG); refs. 1 and 2], various pathological conditions were shown to increase the eCB tone and to inhibit molecular mechanisms that are involved in the production, release, and diffusion of harmful mediators such as proinflammatory cytokines or excess glutamate (3–7). In this issue of PNAS, Witting *et al.* (8) demonstrate that, unexpectedly and contrary to the effects of other brain diseases, cell damage induced by experimental autoimmune encephalomyelitis (EAE), an immune-mediated disease widely used as a laboratory model of multiple sclerosis (MS), does not lead to enhancement of eCB levels, although the cannabinoid receptors remain functional.

Nearly two decades ago, Lyman *et al.* (9) reported that Δ^9-THC, the psychoactive component of marijuana, suppresses the symptoms of EAE. A few years later, Wirguin *et al.* (10) reported the same effect by Δ^8-THC, a more stable and less psychotropic analogue of Δ^9-THC. Thus, THC was shown to inhibit both clinical and histological signs of EAE even before the endocannabinoids were described. THC was also shown to control spasticity and tremor in chronic relapsing EAE, a further autoimmune model of MS (11), and to inhibit glutamate release via activation of the CB_1-cannabinoid receptor in EAE (12). Moreover, mice deficient in the cannabinoid receptor CB_1 tolerate inflammatory and excitotoxic insults poorly and develop substantial neurodegeneration after immune attack in EAE (13).

Multiple lines of evidence implicate the proinflammatory cytokine TNF-α in the pathogenesis of both EAE and MS. Since increased production of IFN-γ and TNF-α were shown to precede clinical manifestation in multiple sclerosis (14), attempts have been made to treat the disease with anti-TNF agents. Recently, Glabinski *et al.* (15) reported that, when given after the onset of clinical signs, treatment with the extracellular domain of the TNF receptor reduced the clinical deficits of the first attack of relapsing–remitting EAE.

Witting *et al.* (8) had previously shown that activation of purinergic $P2X_7$ receptors in inflamed brain increased the production of 2-AG, the most abundant eCB (16–18). They hypothesized that this increase was due to activation of microglia $P2X_7$ receptors by the high levels of ATP spilled by damaged cells. Because microglia and invading brain macrophages express $P2X_7$ receptors under EAE conditions, they now sought to test this hypothesis *in vivo* by measuring brain levels of eCBs in areas of marked cell damage in both wild-type and $P2X_7^{-/-}$ mice. As mentioned above, despite the pronounced cell damage induced by EAE, they did not find increased levels of anandamide and 2-AG (Fig. 1*B*). These results show that, contrary to other types of neuropathies, EAE does not lead to a significant increase in eCB tone, suggesting that this autoimmune disease is associated with a step disrupting eCB production. Because EAE is mediated by primed T cells invading the CNS and releasing large amounts of cytokines, including IFN-γ, the authors examined the effect of IFN-γ on the ability of microglia to produce protective eCBs, and they confirmed that IFN-γ abolished the $P2X_7$ receptor-mediated increase in 2-AG levels. Moreover, induction of EAE in $P2X_7^{-/-}$ mice resulted in even lower eCB levels and more pronounced cell damage than in wild-type mice. These data suggest that the high level of IFN-γ in the CNS, noted in mice with EAE, disrupts eCB-mediated neuroprotection, while maintaining functional cannabinoid receptors, thus providing additional support for the use of cannabinoid-based medicine to treat MS.

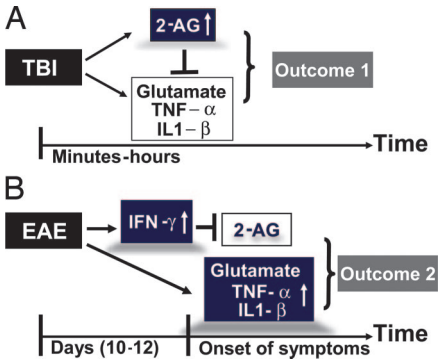

Fig. 1. Traumatic brain injury (TBI) and experimental autoimmune encephalomyelitis (EAE) differentially affect endocannabinoid levels. (*A*) TBI triggers the release of harmful mediators such as glutamate and proinflammatory cytokines, within minutes to hours after injury. Concomitantly, 2-AG is also accumulated, within a similar time frame. 2-AG inhibits, at least in part, the release of glutamate and synthesis of cytokines. The final outcome after injury is determined by the balance between the actions of the harmful and the protective mediators (outcome 1). (*B*) During the development of EAE, IFN-γ is released by primed T cells invading the CNS. IFN-γ inhibits the production of 2-AG; thus, at the time of manifestation of symptoms, the levels of 2-AG are not increased (outcome 2).

Indeed, there are reports on the neuroprotective role of eCB in models of EAE (either acute or chronic relapsing). Jackson *et al.* (19) showed that both neurofilament and myelin basic protein levels decrease over the course of the disease, indicating concomitant neuronal/axonal loss and demyelination. Loss of each marker was more severe in $CB_1^{-/-}$ animals. Active caspase 3 levels, which are increased during EAE, indicating apoptosis, were also more pronounced in $CB_1^{-/-}$ mice. These results show that lack of the CB_1 receptor is associated with greater loss and/or compromise of myelin and axonal/neuronal proteins. Along the same line, Panikashvili *et al.* (20) demonstrated that the $CB_1^{-/-}$ mice did not respond favorably to 2-AG treatment after traumatic brain injury (TBI) in contrast to the wild-type mice. The latter, when treated with exogenous 2-AG, displayed remarkable recovery (4) and inhibition of the brain inflammatory response (20, 21), typically occurring after trauma. Taken together, published data support the hypothesis that endogenous mechanisms of neuroprotection, either after trauma or after EAE, involve, at least in part, CB_1 signaling.

We believe that the differences noted between EAE and other brain pathologies in which the eCB system was studied are due mainly to the nature and time course of these pathologies (Fig. 1). By contrast to the acute traumatic or ischemic brain injury (Fig. 1*A*), EAE is a slowly (10–12 days) progressive disease (Fig. 1*B*). Based on the present study, one may speculate that the difference between the in-

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crease in 2-AG levels after TBI (within hours) and the lack of change noted after the appearance of symptoms in EAE (or the decrease in the $P2X_7^{-/-}$ mice) is due to the enhanced accumulation of IFN-γ taking place during the time preceding the clinical manifestation of symptoms. The accumulation of IFN-γ disrupts the production of eCB while leaving the CB_1 receptor intact.

An earlier report also showed that CB_1 receptor binding and mRNA levels were not affected in EAE rats in brain areas such as the hippocampus, limbic structures, and cerebellum (22). Thus, the brain loses some of its endogenous neuroprotective capacity, but it may still respond to exogenous treatment with 2-AG or other CB_1 agonists. Assuming that the biochemical changes taking place in the EAE model of MS are similar to those in MS itself, these results represent a biochemical-based support to the positive outcome noted with cannabinoid therapy in MS.

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